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Injections of Atropine Into the Caudate Nucleus Impair the Acquisition and the Maintenance of Passive Avoidance

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PRADO-ALCALÁ, R. A., M. FERNÁNDEZ-SAMBLANCAT AND M. SOLODKIN-HERRERA. *Injections of atropine into the caudate nucleus impair the acquisition and the maintenance of passive avoidance.* PHARMACOL BIOCHEM BEHAV 22(2) 243–247, 1985.—Two experiments were performed to test the hypotheses that cholinergic activity of the caudate nucleus (CN) is involved in the acquisition and in the maintenance of passive avoidance behavior. Rats were trained, in one trial, to avoid one of two compartments of a conditioning box and retention of the task was measured 24 hours later. Several doses of atropine were injected into the CN of independent groups of animals. In Experiment 1 the atropine was injected 2 minutes after training and in Experiment 2 it was injected 6 minutes before retention testing. In both cases a dose-dependent retention deficit was found. These results indicate that striatal cholinergic activity is indeed involved in the processes that mediate passive avoidance conditioning.

Caudate nucleus Caudate-putamen Striatum Neostriatum Acetylcholine Atropine
Passive avoidance Learning Retention Conditioning Memory

A series of experimental findings points to the conclusion that passive avoidance behavior in the rat depends, to a large extent, upon the integrity of the caudate nucleus (CN) (for a review see [18]). Other studies suggest that the acquisition of this behavior is mediated by the striatal cholinergic system: cholinergic blockade of the anterior aspect of the caudate-putamen (CPU) induces a marked state of amnesia of the avoidance task [9,22]; furthermore, this amnestic state is time dependent, i.e., a greater impairment in retention is seen as the application of an acetylcholine-receptor blocker is nearer in time to the time of training [26].

To further test the hypothesis that a cholinergic mechanism is involved in the acquisition of passive avoidance, different doses of atropine were injected into the CPU after training. Our results confirm and extend previous reports [9, 22, 26] and new findings are presented which indicate that the striatal cholinergic system is also involved in the maintenance of passive avoidance.

EXPERIMENT 1

In the studies cited above, where anticholinergics were injected into the CPU, only one dose level was used. In order to increase the confidence that the effects that were seen had been due to the blockade of cholinergic receptors of the CPU, different doses of atropine were injected into the striatum of rats shortly after training and retention of the task was tested one day later.

METHOD

Animals

Sixty-two experimentally naive male Wistar rats, weighing between 250 and 350 g were used. They were individually housed and had free access to food and water in their home cages. Each rat was randomly assigned to one of three conditions: with cannulae in the caudate-putamen, with cannulae in the parietal cortex or without cannulae. Under Nembutal anesthesia (50 mg/kg) the double-walled cannulae were bilaterally implanted in the anterior-dorsal aspect of the CPU or in the parietal cortex, overlying the caudate, as described elsewhere [26]. These rats were allowed seven days to recover from the surgical procedures before training was initiated. Two additional groups of rats were not operated upon.

Apparatus

Training and testing were carried out in a box (Lafayette Inst. Co., mod. 85000) with two identical compartments separated by a guillotine door. The grid floor of one of the compartments could be electrified by a square-pulse

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stimulator connected in series with stimulus isolation and constant current units (Grass Med. Inst., mods. S-44, SIU-5A and CCU-1A, respectively).

Procedure

Training and testing. During training each animal was put inside the left-hand side compartment of the conditioning box; ten sec later the door between compartments was opened and the latency to enter to the opposite compartment with all four paws was measured. Once in the second compartment the door was closed and a 1.5 mA footshock (60 msec pulses, 100 pulses per sec) was applied through the grid floor for five sec and the door was reopened, thus allowing the animal to escape to the first compartment and to remain there for 30 sec before being put back in its home cage. There was a group of animals that did not receive the footshock.

Twenty-four hr later a test session was programmed exactly as the training session, except that the footshock was not delivered. If a rat did not cross within 600 sec to the compartment where the footshock had been given the session was ended and a score of 600 was assigned.

Treatments. One of three doses of atropine sulphate (Sigma), dissolved in isotonic saline solution, was injected to independent groups with cannulae implanted in the CPU. The doses were: 20 (n=8), 40 (n=9) and 60 (n=7) μg/3 μL; the group with cortical cannulae was also treated with 60 μg of atropine (n=7). Two additional caudate (control) groups were studied: one was injected with 3 μL of isotonic saline (n=7) and the other one was not injected (n=8). All these groups received a footshock during training, as explained above, as well as a group of unimplanted rats (n=8). The eight group (also unimplanted rats) did not receive the footshock during training (n=8).

The injection procedure was carried out in a room different from the room where training and testing took place. All infusions were bilateral, in a volume of 3 μL through each cannula, delivered two min after training at a rate of 1 μL/20 sec; after injecting the solutions the injectors were left inside the cannulae for an additional min. During the procedure the rats were not restrained by the experimenter and could move freely in their home cages, thus avoiding potential stress-related reactions that could confound the results of the experiment.

Histology. At the conclusion of the experiment all cannulated rats were deeply anesthetized and perfused intracardially with isotonic saline followed by 10% formaline, their brains excised and kept in formaline for at least one week. Coronal sections (50 μm thick) were made and processed (Nissl stain) to determine the location of the cannulae tips.

*Data analyses.*The Kruskal Wallis ANOVA was computed for each session and, when appropriate, the Mann-Whitney U test was used to compare the latency score of each group against the latency score of each of the other groups. A difference in scores was considered to be significant when a p value of less than 0.05 was found (one tailed).

RESULTS AND DISCUSSION

As depicted in Fig. 1, the histological analyses revealed that in the caudate animals the cannulae tips had been lodged in the anterior-dorsal aspect of the CPU, rostral to the last trace of the anterior commissure. The tips of cortical cannulae spanned the same anterior-posterior limits of those of the CPU placements.

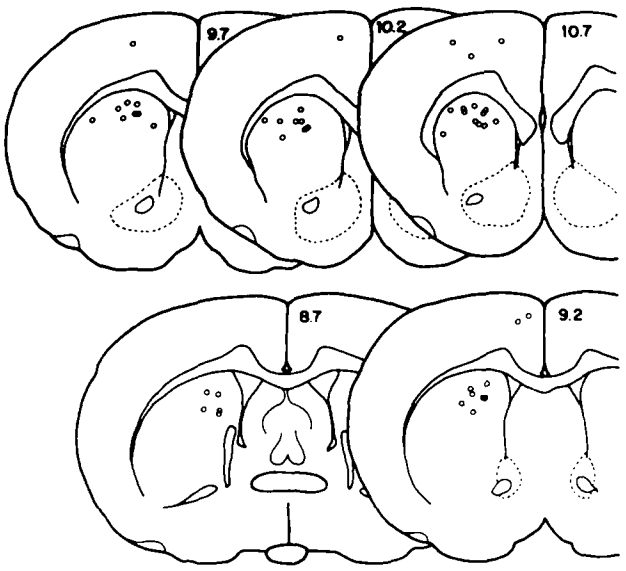

FIG. 1. All cannulae tips were located in the dorsal-anterior aspect of the caudate-putamen, rostral to the last trace of the anterior commissure, or in the cerebral cortex, within the same anterior-posterior limits of the caudate placements. Only the cannulae placements of the right hemisphere are shown. Redrawn from Paxinos and Watson [17].

The analysis of variance indicated that there were no significant differences in latency scores among the groups during training. The retention score for each animal was computed subtracting the latency score of the test session from the latency score of the training session. Highly significant differences among the groups became evident when these retention scores were compared, $H(7)=32.38$, $p<0.001$.

The two main groups against which the rest of the groups were compared (U tests) were the unimplanted-footshocked and the unimplanted-not footshocked groups. There were no significant differences between the former and each of the other caudate control groups (saline injected and not injected groups). The retention of the group that did not receive the footshock did not differ from the retention shown by the caudate group treated with 60 μg of atropine, but differed from the rest of the groups. The retention score of the cortical group, also injected with 60 μg of the anticholinergic drug, lay between those of the not-shocked and of the three control groups, i.e., it differed significantly from each of these groups. A smaller disruptive effect of atropine was seen in the animals injected with 20 or 40 μg into the CPU, as their retention scores did not differ from the cortical nor from the control groups (Fig. 2).

The results of this experiment not only confirm the finding that applications of acetylcholine-receptor blockers into the CPU of rats significantly impair the capacity to learn a passive avoidance task [9, 22, 26] but also show that this impairment is dose-dependent. The group of animals that was injected into the CPU with 60 μg of atropine performed at the same level as the group of intact rats that did not learn the task, namely, the group that did not receive the footshock during training. Those rats injected with 20 or 40 μg of the drug showed retention of the task since their retention scores did not differ significantly from the control groups.

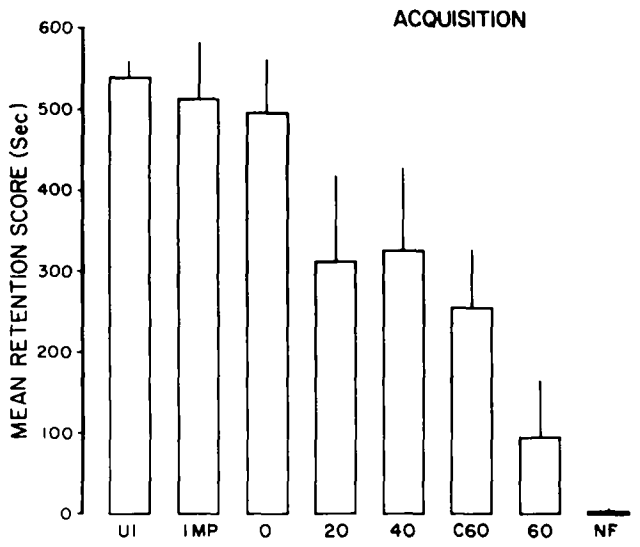

FIG. 2. Mean retention scores (±SEM) shown by the groups tested 24 hours after training. Abbreviations are as follows: UI, unimplanted rats that received a footshock; NF, unimplanted rats that did not receive a footshock; 0, 20, 40 and 60, groups with cannulae implanted in the caudate-putamen and treated with 0, 20, 40 and 60 μg of atropine. C60, group with cannulae implanted in the cerebral cortex, treated with 60 μg of atropine. All implanted animals received a footshock during training and were treated two minutes posttraining.

However, the cortical group treated with 60 μg of atropine did not differ from these two caudate groups, but differed from all other groups. These results indicate that the lower doses of atropine injected into the CPU produced a small retention deficit that would have not been detected if their retention scores had only been compared with the retention of the control groups.

It seems clear that the retention deficits produced by the atropine cannot be attributed to the induction of sensory, motivational, or motoric disturbances since the treatments were given after training had taken place and testing occurred 24 hr later, when the drug had worn off. To put it in another way, all animals were trained and tested in a nondrugged state.

Although these results do not prove by themselves that a cholinergic mechanism within the striatum is critically involved in the acquisition of conditioned responses, several lines of evidence converge to support that hypothesis. First, the chemical machinery necessary for the synthesis of acetylcholine is present in the CN [4, 11, 12, 19] and, furthermore, this cholinergic activity is subserved by intrinsic caudate interneurons [13,14]. Second, changes in acetylcholine metabolism are produced by learning processes: Barker, Glick, Green, and Khandelwal [2] reported that at one hr after training of passive avoidance there was a significant increment in the synthesis of striatal acetylcholine of rats. Along this line, a significant increment in protein synthesis in the CPU was found during the acquisition phase of a continuously reinforced lever-pressing task [1]. Third, experimentally-induced alterations of striatal cholinergic activity bring about changes in the capacity for learning and retention of instrumental tasks. For example, application of choline into the striatum improves the retention of passive avoidance [6] and of lever-pressing [21] behaviors, while application of acetylcholine-receptor blockers impairs the acquisition of both active [16] and passive ([22,26] this report) avoidance and of lever-pressing [3].

With respect to the effect of the injection of atropine into the cerebral cortex, the deficit in retention that was seen had been previously reported [26]. As in the latter case, the retention deficit was smaller than that found after injection of the same dose of atropine (60 μg) into the CPU. This finding suggests that cortical cholinergic activity is also involved in the acquisition of passive avoidance; studies are underway to test this possibility. It is interesting to note that injections of atropine or scopolamine into the same, or equivalent, cortical region explored in the present experiment do not interfere with the performance of positively reinforced behaviors, as they do when injected into the striatum of cats and rats [20,25].

EXPERIMENT 2

To date are no reports on the effects of injections of cholinergic blockers into the CPU on the maintenance of passive avoidance; the results of Experiment 1 only indicate that the acquisition of this aversively motivated behavior is mediated by the striatal cholinergic system. However, there are studies showing that lesions of the CPU impair the performance of passive avoidance [7, 8, 10, 15, 23, 28, 29, 30]. It was of interest, therefore, to explore the possibility that atropine could also interfere with the processes that underly the maintenance (long-term memory) of passive avoidance.

METHOD

Animals

Forty-eight rats of the same characteristics and under the same housing and feeding conditions as in Experiment 1 were used. Cannulae were bilaterally implanted in the anterior-dorsal aspect of the CPU in 32 of these animals.

Apparatus, Procedure, Histology and Statistics

The conditioning box, the training and testing procedures, and the histological and data analyses were the same as in Experiment 1.

Treatments. One of three doses of atropine was injected in each of three groups that had been implanted in the CPU: 20, 60, and 80 μg, respectively, and another caudate group was not injected. Two unimplanted groups were also studied: one of these groups received footshock during training and the last group was the only group that did not receive footshock; there were eight animals in each group. The injection procedures were the same as described in Experiment 1, except that the injections were performed 6 min before the second (test) session, run 24 hr after training.

RESULTS AND DISCUSSION

Before the completion of the experiment two rats lost their cannulae (one from the 60 μg group and the other one from the 80 μg group) and the data yielded by these animals were excluded from the statistical analyses. The cannulae tips of the remaining implanted rats were found to be located, as in Experiment 1, in the antero-dorsal aspect of the CPU. There were no significant differences among the groups when latency scores of the training session were compared. Hence, the difference in scores between the test and acquisition sessions of each rat was used as the retention score.

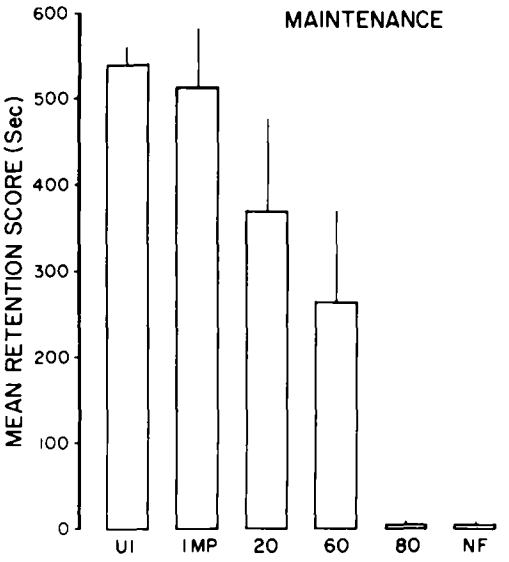

FIG. 3. Mean retention scores (±SEM) shown by the groups tested 24 hours after training. Abbreviations are the same as in Fig. 1; 80 refers to a group treated with 80 μg of atropine. The treatments were delivered six minutes before the retention test.

There were significant differences in retention scores between the groups, $H(7)=28.69$, $p=0.0001$. Pair-wise comparisons between the groups (U tests) showed that there were no reliable differences between the untrained (not-footshocked) group and the group treated with 80 μg of atropine. Each of these two groups had significantly lower retention scores than the rest of the groups. No differences appeared among the rest of the groups, except between the animals treated with 60 μg of atropine and the implanted-not injected animals (Fig. 3).

This is the first report where a dose-dependent impairment in the maintenence of passive avoidance is shown; this result gives further support to the hypothesis that cholinergic activity of the caudate nucleus is involved in the mechanisms responsible for long-term retention of instrumental behaviors.

The dose-dependent impairment in retention suggests that there is a critical number of cholinergic synapses that must be blocked in order to observe an amnestic state: the lowest dose of atropine (20 μg) did not produce a retention deficit, the intermediate dose (60 μg) produced a loss of memory of about 50% as compared with the control groups that received a footshock (unimplanted and implanted-not injected animals), and the highest dose (80 μg) induced a complete lack of retention.

The possibility that the impairments seen after the atropine injections were due to interference with non-mnemonic processes warrants consideration. First, these results could be explained by a state-dependent effect since the treated animals were trained in a non-drugged state and tested under the influence of the atropine. Two lines of evidence argue against this possibility, however: (a) when rats are trained in the same task reported here, injected with atropine into the caudate two min later, and then tested for retention 30 min afterwards, while still under the effects of the drug, there are no retention deficits [27], and (b) when rats are trained, tested and treated as in this Experiment, but are given a stronger footshock, they show as good retention as untreated animals [5]. Second, the atropine could have produced a state of hypermotility and this could have been the cause for the low latencies displayed during the test session by the groups treated with 60 and 80 μg. Although this possibility cannot be completely ruled out, consideration of other experimental findings lead us to discard such possibility.

The effects of both atropine and scopolamine injections into the CPU on conditioned behaviors that are expressed as a facilitation of motor activity, such as active avoidance, lever pressing and alley-running have been tested [16, 20, 24, 25]. It has been found that the performance of those behaviors is impaired, i.e., there is a decrement in motor output. In the case of passive avoidance, where the learned response is expressed as a suppression of motor activity, application of anticholinergic drugs into the CPU also produces an impairment in performance, in this case reflected as an increment in motor output [9, 22, 26]. If the effect of the anticholinergics were due to a disturbance in motor activity (hyperactivation or hypoactivation) then one would expect to find an improvement in one type of learned behavior and a deficit in the other. However, regardless of the type of motor activity involved, the common feature of the effects of cholinergic blockade of the caudate nucleus is a loss of the learned response.

To conclude with this point, it is pertinent to mention a recent experiment where footshocks of high intensity were used in a passive avoidance task very similar to the one described here; the injection of scopolamine (30 μg) or atropine (60 μg) into the CPU, six min before the test session or two min after training, did not produce a retention deficit, i.e., there was not an increment in locomotor activity [5]. These results also show that these drugs did not produce any significant alterations in other variables involved in the performance of the task, such as motivational or perceptual processes.

Taken together, the data reviewed above and the findings reported in this paper further support the hypothesis that cholinergic activity of the caudate nucleus is critically involved in the acquisition and maintenance of instrumental behaviors, in general, and of passive avoidance, in particular.

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